The 35 U.S.C. §112 Rejection

Claims 10-13, 15-17 were rejected under 35 U.S.C. §112, first paragraph, for lack of enablement. The rejection is respectfully traversed.

The Examiner has required a deposit of a mutant strain that contains a mutated recA gene before the claims will be allowed. Applicants submit that that the appropriate deposit will be made prior to allowance of the claims. Accordingly, Applicants respectfully request that the rejection of claims 10-13, 15-17 under 35 U.S.C. §112, first paragraph, be held in abeyance.

Claim 18 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite. The rejection is traversed.

Claim 18 has been amended to recite a mutant strain that contains more than one altered *sap*A homolog but only one unaltered *sap*A homolog. Applicants submit that the claim has particularly pointed out and claimed the subject matter. Accordingly, Applicants respectfully request that the rejection of claim 18 under 35 U.S.C. §112, second paragraph, be withdrawn.

The 35 U.S.C. §102 Rejection

Claims 1, 6-8 were rejected under 35 U.S.C. §102(a) as being anticipated by **Dworkin** et al. (March 1996). The rejection is respectfully traversed.

The Examiner stated that Applicants' arguments filed with respect to **Dworkin** et al. were not persuasive because of the disclosure in Figures 3a, 3b, 7 and Table 1 of **Dworkin** et al. However, in the Office Action mailed April 27, 2000, the Examiner only provided Applicants with an abstract, not the full paper, of **Dworkin** et al. (March 1996). Therefore, Applicants do not have Figures 3a, 3b, 7 and Table 1 of **Dworkin** et al. to respond to the Examiner's argument.

In the instant Office Action, Applicants' argument was rejected by the Examiner because **Dworkin** et al. taught mutant strains that have not been structurally mutated. Applicants submit that the Examiner's argument on structurally non-mutated bacteria is irrelevant to claims 1 and 6-8. Claims 1 and 6-8 are drawn to a genetically engineered mutant strain, not a structurally non-mutated strain, and it is unclear how teaching on non-mutated bacteria would anticipate the claims on mutated bacteria.

Claim 1 is drawn to a mutant strain of *Campylobacter fetus* that contains a DNA cassette encoding a heterologous protein, wherein the insertion of said DNA cassette results in alteration of a *sapA* homolog, and the expression of said DNA cassette results in a S-layer protein that represents a chimera between the native S-layer protein and the heterologous protein encoded by said DNA cassette. **Dworkin** et al. (March 1996) did not teach or suggest insertion of a DNA cassette encoding a foreign heterologous protein alters a *sapA* homolog, nor did **Dworkin** et al. teach or suggest the expression of said DNA cassette results in a chimera between the native S-layer protein and the heterologous protein encoded by said DNA cassette. Since a claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference, and the identical invention must be shown in as complete detail as is contained in the claim, claim 1 of the instant invention is not anticipated by **Dworkin** et al.

Claim 1 of the present invention is drawn to a genetically engineered mutant *C. fetus* strain expressing one or more altered *sap*A homologs on cell surface, each of said altered *sap*A homolog is altered by inserting a heterologous protein into the

coding sequence of said *sap*A homolog. **Dworkin** et al. (March 1996) did not teach or suggest insertion of a foreign heterologous protein would result in surface expression of an altered *sap*A homolog as claimed herein. **Dworkin** et al. teach loss of cell surface S-layer expression due to insertion of a foreign heterologous protein kanamycin (Figure 1, K100 and K300 series; Figure 2) because "insertion of km at base pair 127 of the SLP gene cassette ORF was known to interrupt SLP transcription" (page 1242, right column, lines 21-22). Hence, **Dworkin** et al. indeed teach away from the present invention, and claim 1 of the instant invention is not anticipated by **Dworkin** et al.

Claim 8 is drawn to a mutant *Campylobacter fetus* strain of claim 1 that expresses a heterologous protein such as an antigen or a therapeutic agent. **Dworkin** et al. did not teach or suggest a mutant strain that expresses foreign heterologous protein as claimed herein. Since "the identical invention must be shown in as complete detail as is contained in the ... claim." (Fed. Cir. 1989), **Dworkin** et al. does not anticipate claim 8 of the present invention. Accordingly, Applicants respectfully request that the rejection of claims 1, 6-8 under 35 U.S.C. §102(a) be withdrawn.

Claims 1, 6-8 were rejected under 35 U.S.C. §102(b) as being anticipated by **Blaser** et al. (November 1994). The rejection is respectfully traversed.

In the Office Action mailed April 27, 2000, the Examiner only provided Applicants with an abstract of **Blaser** et al. (November 1994). In the abstract of **Blaser** et al. (November 1994), it is disclosed that "disruption of sapA by a gene targeting method (insertion of kanamycin resistance) caused the loss of *C. fetus* cells bearing full-length S-layer proteins and their replacement by cells bearing a 50 kDa truncated protein that was not exported to the cell surface" (lines 5-9). In contrast, the present invention is drawn to a mutant *C. fetus* strain expressing one or more altered *sap*A homologs on cell surface, each of said altered *sap*A homolog is altered by insertion of a DNA cassette that encodes a heterologous protein. Hence, the claims in the instant invention are different and distinct from that of **Blaser** et al. Since **Blaser** et al. clearly did not teach or suggest each and every aspect of the present invention, **Blaser** et al. did not anticipate the present invention. Accordingly, Applicants respectfully request that the rejection of claims 1, 6-8 under 35 U.S.C. §102(b) be withdrawn.

The 35 U.S.C. §103(a) Rejection

Claims 1, 5-9 were rejected under 35 U.S.C. §103(a) as being unpatentable over **Blaser** (1994) in view of **Lubitz** et al. The rejection is respectfully traversed.

As discussed above, **Blaser** et al. (1994) teaches away from the present invention in that **Blaser** (1994) disclosed the insertion of a kanamycin cassette into sapA results in a truncated protein that was not exported to the cell surface. In contrast, the instant invention is drawn to a mutant *C. fetus* strain that expresses altered sapA homolog(s) on the surface of the bacteria.

The Examiner argued **Blaser** et al. did not teach away from the present invention by asserting that **Blaser** et al. taught mutant strains that reverted to express *sap*A and retented the heterologous antigen kanamycin. However, **Blaser** et al. did not teach surface expression of altered *sap*A homolog as claimed herein. **Blaser** et al. only taught reversion that expressed wild type *sap*A (abstract, lines 11-12), not altered or mutated *sap*A homolog as claimed herein. What is clearly shown in **Blaser** et al. is that insertion of a heterologous antigen kanamycin resulted in modified

*sap*A that cannot be expressed on cell surface, and certain mutants could revert to express wild type *sap*A on cell surface due to a mechanism of homologous recombination that causes antigenic variations in the bacteria (abstract, lines 16-19).

The Examiner argued that **Lubitz** et al. did not teach away from the present invention and **Lubitz** taught the incorporation of an immunogen into the S-layer of the bacteria (col. 2, lines 24-26; Figure 2d). Applicants strongly disagree. Applicants submit that **Lubitz** et al. did not teach or suggest incorporation of an immunogen into the S-layer of the bacteria as claimed herein.

Lubitz only taught the use of bacterial ghosts to carry immunogens by expressing a fusion protein that inserts into the bacterial membrane, not into the S-layer coat which is outside the outer membrane of the bacteria (column 2, lines 5-19, 56-58). Figure 2 in **Lubitz** et al. depicted a longitudinal section of a bacterial ghost, showing insertion of fusion protein into the inner or outer membrane of the bacteria (Figure 2d). Figure 2 did not show insertion of fusion protein into the S-layer coat which is outside the outer membrane of the bacteria. The Examiner's assertion that Figure 2d showed incorporation of an immunogen into the S-layer is

clearly wrong because Figure 2d did not even show the S-layer coat of the bacteria.

If the bacteria possess an additional S-layer coat, the S-layer is also a component of the bacteria ghost, but the S-layer coat with its lipopolysaccharide only serves to enhance the immune responses to the antigens (column 2, lines 23-31; column 3, lines 2-6), not as a site for insertion of heterologous fusion protein. Therefore, **Lubitz** et al. did not teach or suggest S-layer being carrier proteins for heterologous antigens, nor did **Lubitz** teach or suggest modification of the S-layer or the sap homologs in *C. fetus* as claimed herein.

The Examiner's arguments for combining **Blaser** et al. and **Lubitz** et al. are illogical and unreasonable. First of all, the Examiner argued that **Blaser** et al. "clearly is evaluating antigenic characteristics of the strains, virulence characteristics and the immune response stimulated in the host through challenge experiments using the mutant strains." Applicants agreed that **Blaser** et al. examined the antigenic and virulence characteristics of the mutant *C. fetus* strains by immunoblot and bacteremia study respectively, but **Blaser** did not teach or suggest any evaluation or

measurement of immune response to *C. fetus* in a mammalian host. **Blaser** et al. did not have any disclosure on simple and basic questions of immune responses such as antibody secretion stimulated by the bacteria in a host, or whether immunity was induced in a host so that the host would resist infection upon subsequent encounter with the bacteria. Applicants reiterate that bacteremia study is used to determine the virulence of the bacteria, not the immune responses against the bacteria. **Blaser** et al. simply did not provide a clear and sufficient disclosure on immune responses against the mutant bacteria. In view of the lack of relevant disclosure in **Blaser** et al., one of ordinary skill in the art would not has the slightest hint on the nature and extend of immune responses against the mutant bacteria.

Secondly, the Examiner argued that "**Blaser** clearly teaches the use of *C. fetus* as a strain that is useful for the evaluation of the immune system of a mammalian host and in evaluating the immune response to antigens expressed (immunoblot)." However, one of ordinary skill in the art would not use immunoblot to evaluate immune response to antigens. **Blaser** used immunoblot to determine the size of full-length S-layer

proteins expressed in mutant *C. fetus* strains (abstract, lines 9-12). Thus, the immunoblot was used to determine the antigenic characteristic of mutant *C. fetus* strains, not immune responses to the bacteria. One of ordinary skill in the art would readily recognize that the immunoblot assay is simply not the appropriate assay for immune responses evaluation becasue the immunoblot did not measure any immune response to *C. fetus* in a host.

Thirdly, the Examiner argued that "both Blaser and Lubitz teach and suggest the evaluation of expressed heterologous antigens, as well as the construction of mutant strains of bacteria that comprise DNA cassettes that comprise a heterologous antigen." However, as discussed above, **Blaser** et al. taught a mutant construction that cannot be expressed on cell surface, whereas **Lubitz** et al. did not teach or suggest inserting an altered bacterial protein into the S-layer of the bacteria. In contrast, the main feature of the instant invention is the construction of modified *C. fetus* that express altered S-layer proteins on the surface of the bacteria. Hence, it is unreasonable and illogical that the present invention would be derived from combining the teaching of **Blaser** and **Lubitz** that (1) did not teach or suggest making S-layer

chimera which is expressed on cell surface, and (2) did not teach or suggest modification involving the S-layer.

In view of the above remarks, the combined teaching of **Blaser** et al. and **Lubitz** et al. does not provide a person having ordinary skill in this art with the requisite expectation of successfully producing Applicants' claimed invention. The invention as a whole is not *prima facie* obvious to one of ordinary skill in the art at the time the invention was made. Accordingly, Applicants respectfully submit that the rejection of claims 1, 5-9 under 35 U.S.C. §103(a) be withdrawn.

New Ground Of Rejection

Claims 15-17 were rejected under 35 U.S.C. §112, first paragraph, for lack of possession of the claimed invention. The rejection is respectfully traversed.

The Examiner required a deposit of the genetic material containing the claimed *sapCDEF* genes. Applicants submit that the appropriate deposit will be made. Accordingly, Applicants

respectfully request that the rejection of claims 15-17 under 35 U.S.C. §112, first paragraph, be withdrawn.

Claims 1, 5-13, 15, 16 and 18 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite. The rejection is respectfully traversed.

Claim 1 was rejected for lack of clarity. Claim 1 has been amended to recite a genetically engineered mutant *C. fetus* strain expressing one or more altered *sap*A homologs on cell surface. The altered *sap*A homolog(s) is altered by inserting a DNA cassette that encodes a heterologous protein into the coding sequence of said *sap*A homolog.

Claim 5 has been amended as helpfully suggested by the Examiner, reciting "said heterologous protein is an immunogen".

Claim 6 was rejected for lack of clarity. Claim 6 has been amended to recite a DNA cassette comprises a 5' LPS-binding region of said *sap*A homolog, a 3' secretion signal region of said *sap*A homolog and sequence encoding said heterologous protein inserted between said binding region and said signal region.

Claim 7 has been amended to clearly define the DNA cassette. The DNA cassette contains a 3' secretion signal of a *sap*A homolog and sequence encoding a heterologous protein inserted upstream of said secretion signal, but the DNA cassette does not has a 5' LPS-binding region of a *sap*A homolog.

The Examiner questioned the difference between antigen and therapeutic agent recited in claim 8. Applicants submit that one of ordinary skill in the art would readily recognize the difference between antigen and therapeutic agent. To one of ordinary skill in the art, antigen refers to a molecule that can be used to induce an immune response, whereas therapeutic agent is a substance that is useful in treating disease or wound.

Claim 9 was rejected for reciting immunogen without antecedent basis. Claim 9 has been amended to be dependent from claim 5 which provides the antecedent basis.

Claim 10 was rejected for not clearly defining the mutant strain. Claim 10 has been amended to recite a mutant *C. fetus* strain expressing only one S-layer protein encoded by one *sap*A homolog due to a *recA* mutation that results in no functional RecA protein expression.

Claim 11 was rejected for lack of clarity. Claim 11 has been amended to recite a mutant bacteria that contains a chimeric protein comprising a heterologous antigen and a *sap*A homolog.

Claim 12 was rejected for lack of clarity. Claim 12 has been amended to recite a mixture of mutant *C. fetus* strains, wherein due to *recA* mutation that results in no functional RecA protein expression in each of said strain, each of said strain expresses only one S-layer protein comprising a heterologous antigen and a *sap*A homolog.

Claim 15 was rejected for not reciting the full name of *SapCDEF* and the source of *SapCDEF* . Claim 15 has been amended to recite the full name of *SapCDEF* and the source of *SapCDEF* .

Claim 16 was rejected for lack of clarity. Claim 16 has been amended to distinctly identify and claim the sequences that encode a chimeric protein in the modified bacteria.

Claim 18 was rejected for reciting a phrase without antecedent basis. Claim 18 has been amended to recite a mutant strain that comprises more than one altered *sap*A homolog and only one unaltered *sap*A homolog. Applicants submit that the claims have been amended to particularly pointed out and claimed the subject

matter of the instant invention. Accordingly, Applicants respectfully request that the rejection of claims 1, 5-13, 15, 16 and 18 under 35 U.S.C. §112, second paragraph, be withdrawn.

This is intended to be a complete response to the Office Action mailed September 27, 2001. If any issues remain outstanding, the Examiner is respectfully requested to telephone the undersigned attorney of record for immediate resolution.

Respectfully submitted,

Date: Jan 16, 2002

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Claim 1 has been amended as follows:

1. (thrice amended) A genetically engineered mutant *C. fetus* strain ~~useful for vaccinating an animal to *Campylobacter fetus*, wherein said strain is mutated to contain a DNA cassette encoding a heterologous protein, wherein the insertion of said DNA cassette results in alteration of a *sapA* homolog, and the expression of said DNA cassette results in surface expression of a S layer protein that represents a chimera between the native S layer protein and the heterologous protein encoded by said DNA cassette.~~ expressing one or more altered *sap*A homologs on cell surface, each of said altered *sap*A homolog is altered by inserting a DNA cassette that encodes a heterologous protein into the coding sequence of said *sap*A homolog.

Claim 5 has been amended as follows:

5. (twice amended) The mutant *C. fetus* strain of claim 1, wherein said heterologous protein is an immunogen ~~DNA cassette encodes immunogens~~ of a pathogen~~, wherein said pathogen~~

~~is~~ selected from the group consisting of *Salmonella*, *Shigella*, *Campylobacter jejuni*, *E. coli* 0157:H7, human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV) and animal pathogens.

Claim 6 has been amended as follows:

6. (amended) The mutant *C. fetus* strain of claim 1, wherein said DNA cassette comprises ~~contains~~ a 5' LPS-binding region of said *sap*A homolog, a ~~and~~ 3' secretion signal region of said *sap*A homolog and ~~wherein~~ sequence encoding said heterologous protein ~~is~~ inserted between said binding region and said signal region.

Claim 7 has been amended as follows:

7. (amended) The mutant *C. fetus* strain of claim 1, wherein said DNA cassette comprises ~~contains~~ a 3' secretion signal of said *sap*A homolog and sequence encoding said heterologous protein inserted upstream of said secretion signal, but said DNA cassette does not has a 5' LPS- ~~no~~ binding region of said *sap*A homolog.

Claim 9 has been amended as follows:

9. (amended) A method of immunizing a host to develop mucosal and systemic immune responses to an immunogen carried by the mutant strain of claim 5, comprising the step of administering to said host a pharmacologically effective dose of the strain of claim 5 ~~1~~.

Claim 10 has been amended as follows:

10. (amended) A mutant *C. fetus* strain, ~~wherein *recA* is mutated so that no functional RecA protein is produced, the DNA rearrangements permitting *sapA* antigenic variation occur at a very low frequency and wherein said *C. fetus* strain can only produce one of the S-layer proteins encoded by one *sapA* homolog.~~ expressing only one S-layer protein encoded by one *sap*A homolog due to a *recA* mutation that results in no functional RecA protein expression.

Claim 11 has been amended as follows:

11. (amended) The mutant *C. fetus* strain of claim 10, wherein said strain contains a ~~*sapA* homolog expressing a~~ chimeric protein comprising ~~including~~ a heterologous antigen and a *sap*A homolog.

Claim 12 has been amended as follows:

12. (amended) A mixture of mutant *C. fetus* strains, ~~wherein each strain includes a *sapA* chimera which is also a *recA* mutant, wherein a single *sapA* homolog is mutated to encode a different chimeric protein representing a different heterologous antigen and each mutant is also RecA deficient due to mutation in *recA*.~~ wherein due to *recA* mutation that results in no functional RecA protein expression in each of said strain, each of said strain expresses only one S-layer protein comprising a heterologous antigen and a *sap*A homolog.

Claim 13 has been amended as follows:

13. (amended) A method of immunizing a host to develop mucosal and systemic immune responses to the antigens of

claim 12 ~~an immunogen~~, comprising the step of administering to said host a pharmacologically effective dose of the strains of claim 12.

Claim 15 has been amended as follows:

15. (twice amended) A strain of *Escherichia coli.* modified to express ~~*SapCDEF* genes.~~ the surface array proteins C, D, E and F of *C. fetus.*

Claim 16 has been amended as follows:

16. (twice amended) The *Escherichia coli.* of claim 15, ~~wherein a heterologous protein is expressed as a chimeric protein composed of sequences of heterologous origin, sequences that direct the secretion of said chimeric protein to the cell surface and sequences that direct the binding of the secreted chimeric protein to the lipopolysaccharides of the bacterial cell surface via the sapCDEF directed type 1 secretory system.~~ further comprising a chimeric protein encoded by sequences comprising a 5' LPS-binding region of a *sap*A homolog, a 3' secretion signal region of a *sap*A homolog and

sequence encoding a heterologous protein inserted between said binding region and said signal region.

Claim 18 has been amended as follows:

18. (twice amended) The mutant *C. fetus* strain of claim 1, wherein ~~all but one of the seven to nine *sapA* homologs are altered due to the insertion of said DNA cassette.~~ said mutant strain comprises more than one altered *sap*A homolog and only one unaltered *sap*A homolog.